# Effects of Aeration and Storage Temperature on *Campylobacter* Concentrations in Poultry Semen[1]

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**ABSTRACT** *Campylobacter* is one of the most commonly reported bacterial causes of human foodborne infections in the United States. Recent evidence has demonstrated that *Campylobacter* is present in poultry semen and may contribute to the vertical transmission between the breeder hen and offspring. As *Campylobacter* is considered sensitive to oxygen and cold temperature, the objective of this study was to determine if aeration and storage temperature could reduce or eliminate *Campylobacter* in poultry semen. In 4 separate trials, pooled semen samples were collected from roosters or toms, diluted with a commercial poultry semen extender, and inoculated with an average of $10^7$ cells/mL of a wild-type *C. jejuni* or *C. coli* semen isolate. Pooled ejaculates were then divided into 3 aeration treatments: Control (no aeration), air, or oxygen (gently bubbled for 20 min with atmospheric air or oxygen, respectively). Immediately after aeration, pooled semen samples were further divided to 3 test storage temperatures: 4, 23, or 42°C. At 0, 2, 6, and 24 h of storage, samples were enumerated for *Campylobacter*. Aeration of the semen alone, or aeration with reduced temperatures (4 or 23°C), did not reduce the amount of *Campylobacter* compared with the controls. *Campylobacter* concentrations were, however, reduced when stored at 42°C for 24 h. This effect is associated with reduced sperm viability and is therefore not a practical treatment of reducing *Campylobacter* in semen. It appears alternative methods will be needed to eliminate *Campylobacter* from poultry semen.

(*Key words*: aeration, *Campylobacter*, chicken, semen, turkey)

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## INTRODUCTION

*Campylobacter* is the one of the most commonly reported bacterial causes of human foodborne infections in the United States (Friedman et al., 2000; CDC, 2002). Epidemiological evidence indicates that a significant proportion of human infections result from the improper preparation of poultry products (Jacobs-Reitsma, 2000; Corry and Attabay, 2001). Although a substantial number of retail chicken and turkey products are contaminated with *Campylobacter* (Norkrans and Svedhem, 1982; Genigeorgis et al., 1986; Shane, 1992; Zhao et al., 2001), the mechanisms by which poultry flocks become infected with this organism are not fully understood.

Many studies suggest that horizontal transmission from environmental sources is the primary route of *Campylobacter* infection (Jacobs-Reitsma, 1997; Sahin et al., 2002). Recent research, however, has demonstrated the vertical transmission of *Campylobacter* between broiler breeder flocks and their offspring (Cox et al., 2002a; Hiett et al., 2002). It has been demonstrated that *Salmonella*, another foodborne pathogen, can be transferred from parent flocks to their progeny through the transovarian route (Baker et al., 1980; McGarr et al., 1980; Timoney et al., 1989; Shivaprasad et al., 1990; Reiber et al., 1995). Furthermore, semen may serve as the vehicle for transmission to the hen and subsequent eggs (Reiber et al., 1991).

Bacterial contamination is highly prevalent in poultry semen (Reiber et al., 1995) with reports of an average of 2.2 million bacteria/mL in chicken semen (Wilcox and Shorb, 1958) and 1.3 billion bacteria/mL in turkey semen (Gale and Brown, 1961). The most frequently isolated bacteria in chicken semen have been *Escherichia*, *Staphylococcus*, *Micrococcus*, *Enterococcus*, and *Salmonella* (Reiber et al., 1995). Recently, *Campylobacter* has been isolated in the semen of commercial broiler breeder roosters (Cox et al., 2002b) and commercial toms (Donoghue et al., 2004).


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**Abbreviation Key:** CEB = *Campylobacter* enrichment broth; CLA = *Campy*-Line agar.

Semen on commercial turkey farms, and some commercial broiler breeder facilities, is routinely pooled and used to inseminate multiple hens, and therefore is a potential source of *Campylobacter* contamination in the female reproductive tract and fertile eggs. This idea is supported by the recent findings of Buhr et al. (2002), who identified *Campylobacter* in the reproductive tract of broiler breeder hens.

*Campylobacter* is a fastidious, thermophilic organism that prefers microaerophilic conditions for growth (Bolton and Coates, 1983; Koidis and Doyle, 1983; Ketley, 1997). Many studies have shown that *Campylobacter* can be reduced or eliminated by exposure to cold temperatures and aerobic environments (Doyle and Roman, 1981; Bolton and Coates, 1983; Kazwala et al., 1990; Hazeleger et al., 1998; Kelana and Griffiths, 2003). Theoretically, aeration and lowering the temperature of semen (or both) should have a similar effect on *Campylobacter* in poultry semen. Furthermore, these treatments should not adversely affect the fertility of the semen (Donoghue and Wishart, 2000). Researchers have demonstrated that aeration of poultry semen promotes the viability of turkey sperm when stored in vitro (Sexton, 1974; Wishart, 1981). Aeration can be accomplished by agitating diluted semen on an orbital shaker (Christensen, 1995) or by bubbling aeration gases through the diluted semen (Thurston et al., 1994). The optimum temperature for maintaining the viability of turkey sperm during in vitro liquid storage ranges from 4 to 15°C (Bajpai and Brown, 1964; Sexton, 1982; Blesbois and Mauger, 1989; Christensen, 1995); such temperatures have been shown to be detrimental to the growth and survival of *Campylobacter* (Kelana and Griffiths, 2003). Therefore, the objective of this study was to determine if aeration and various storage temperatures could reduce or eliminate *Campylobacter* in poultry semen.

## MATERIALS AND METHODS

### Semen Collection

Semen samples from broiler breeder roosters and commercial toms were collected by abdominal massage (Burrows and Quinn, 1937) and aspirated into sterile test tubes. Care was taken to reduce fecal contamination during semen collection by wiping the phallus first. In 4 separate trials, semen samples were collected from roosters and toms, pooled within species, and subjected to the following procedures.

### Determination of Endogenous *Campylobacter* Concentrations in Semen

To determine the endogenous *Campylobacter* concentrations of the pooled semen samples, raw semen was taken from each pooled sample and assessed using standard bacteriological methods with slight modifications (Cox et al., 2002b). Briefly, 0.1 mL of raw semen was diluted with 0.9 mL of *Campylobacter*-enrichment broth (CEB), 10-fold serial dilutions in CEB were performed, and 0.1 mL of each dilution was plated on *Campy*-Line agar (CLA; Line, 2001). Plates were incubated at 42°C for 48 h in a microaerophilic environment (5% $O_2$, 10% $CO_2$, and 85% $N_2$). After incubation, characteristic colonies were confirmed as *Campylobacter* by observation of typical cellular morphology using phase contrast microscopy and by using a commercial latex agglutination test kit[3] specific for *C. jejuni*, *C. coli*, and *C. laridis*. The colonies on each CLA plate were counted on a Leica Darkfield Plate Colony Counter[4] and the direct counts were converted to $\log_{10}$ colony forming units per milliliter of pooled semen.

### Inoculation of Semen with Campylobacter

Before allocating samples to aeration and temperature treatments, semen was inoculated with *Campylobacter*. Each semen sample was diluted 1:4 (vol/vol) with Field Ready Green Extender[5] (without antibiotics) and thoroughly mixed. A 0.1-mL sample was taken from each semen sample and serially diluted with CEB. The dilutions were plated on CLA and enumerated as previously described. The pooled chicken and turkey semen samples were then inoculated with 1.0 mL of CEB averaging $3.1 \times 10^7$ cells/mL of a wild-type *C. jejuni* semen isolate, 1.0 mL of CEB averaging $8.3 \times 10^7$ cells/mL of a wild-type *C. coli* semen isolate, or 1.0 mL of CEB alone (control). These wild-type isolates were collected previously from either chicken or turkey semen (Donoghue et al., 2004). *Campylobacter jejuni* was used to inoculate the pooled chicken semen samples as it is the most prevalent *Campylobacter* species isolated in retail chicken products (Nielsen and Nielsen, 1999), whereas *C. coli* was used to inoculate the pooled turkey semen, as it is more frequently isolated from retail turkey products (Zhao et al., 2001).

### Aeration of Semen

Following inoculation with *Campylobacter*, the semen samples were divided equally into 3 sterile test tubes according to treatment group: Control – no aeration, oxygen – gentle bubbling for 20 min with oxygen, or air – gentle bubbling for 20 min with atmospheric air (Thurston et al., 1994). The air treatment test tubes were aerated using an aquarium pump.[6] Air flowed from the aquarium pump into the semen through a 60-cm plastic tube (0.5 cm diameter) and sterile glass pipette whose tip extended to just above the bottom of the test tube (Thurston et al., 1994). The oxygen treatment test tubes were aerated using compressed pure oxygen that flowed into the semen through a 60-cm plastic tube (0.5 cm diameter) and sterile glass pipette whose tip extended just above the bottom of the test tube. Immediately following aeration (0 h), the semen samples were further divided into 3 test storage temperature groups: 4, 23, or 42°C. As mentioned pre-

[3]Pan Bio, Inc., Columbia, MD.
[4]Leica Inc., Buffalo, NY.
[5]IMV International Corp., Maple Grove, MN.
[6]Aqua Culture, Kuala Lumpur, Malaysia.

**TABLE 1. Effects of aeration and storage temperature on *Campylobacter* concentrations (cfu/mL) in chicken semen[1]**

| | Control | | | Air | | | Oxygen | | |
|---|---|---|---|---|---|---|---|---|---|
| Time | 4°C | 23°C | 42°C | 4°C | 23°C | 42°C | 4°C | 23°C | 42°C |
| Pretreatment | NA | $7.7 \times 10^{6,a}$ | NA | NA | $7.7 \times 10^{6,a}$ | NA | NA | $7.7 \times 10^{6,a}$ | NA |
| 0 h | NA | $9.3 \times 10^{6,a}$ | NA | NA | $3.8 \times 10^{6,a}$ | NA | NA | $6.4 \times 10^{6,a}$ | NA |
| 2 h | $9.7 \times 10^{6,a}$ | $7.1 \times 10^{6,a}$ | $8.1 \times 10^{6,a}$ | $5.9 \times 10^{6,a}$ | $1.1 \times 10^{6,a}$ | $7.1 \times 10^{6,a}$ | $7.6 \times 10^{6,a}$ | $5.3 \times 10^{6,a}$ | $8.9 \times 10^{6,a}$ |
| 6 h | $6.8 \times 10^{6,a}$ | $7.8 \times 10^{6,a}$ | $2.3 \times 10^{7,a}$ | $6.2 \times 10^{6,a}$ | $7.1 \times 10^{6,a}$ | $1.9 \times 10^{7,a}$ | $7.9 \times 10^{6,a}$ | $5.1 \times 10^{6,a}$ | $2.4 \times 10^{7,a}$ |
| 24 h | $6.4 \times 10^{6,a}$ | $2.9 \times 10^{6,a}$ | $1.0 \times 10^{2,b}$ | $4.7 \times 10^{6,a}$ | $5.3 \times 10^{6,a}$ | $1.5 \times 10^{2,b}$ | $1.6 \times 10^{6,a}$ | $7.0 \times 10^{5,a}$ | $3.8 \times 10^{2,b}$ |

[a,b]Means with no common superscript differ significantly ($P < 0.05$). All data were $\log_{10}$ transformed for statistical analysis. For clarity of presentation, arithmetic means are presented.

[1]Means of 4 separate trials. In each trial, pooled chicken semen was diluted with Field Ready Green Extender (without antibiotics) and inoculated with 1.0 mL of *Campylobacter* enrichment broth containing an average of 3.1 x $10^7$ cells/mL of a wild-type *C. jejuni* semen isolate. Following inoculation, pooled semen was gently bubbled with atmospheric air using an aquarium pump or compressed oxygen for 20 min, or not aerated (control). Immediately following the 20 min aeration/control period (0 h), semen samples were allocated to the 4, 23, or 42°C treatment groups. NA = not applicable.

viously, 4°C and 23°C are temperatures commonly used for long- and short-term liquid storage of turkey semen, respectively (Sexton, 1988). The optimum growth temperature of *Campylobacter* is 42°C (Ketley, 1997), and this temperature was therefore used as a *Campylobacter* growth control. At 0, 2, 6, and 24 h of storage, a 0.1-mL sample was taken from each aliquot and serially diluted with CEB. The dilutions were plated on CLA and evaluated for *Campylobacter* as previously described. Sperm viability was assessed for each treatment group at the end of each trial according to the method of Wishart and Wilson (1997).

### Statistical Analysis

Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, 1994). The numbers of *Campylobacter* colonies were logarithmically transformed ($\log_{10}$ cfu/mL) before analysis to achieve homogeneity of variance (Byrd et al., 2003). Treatment means were partitioned by LSMEANS analysis (SAS Institute, 1994). A probability of $P < 0.05$ was required for statistical significance. The data in Tables 1 and 2 are shown as arithmetic means for clarity of presentation.

## RESULTS

Before inoculating the semen samples with *Campylobacter*, endogenous concentrations of the organism ranged from $<10^2$ (below the limit of detection) to $3.7 \times 10^3$ cfu/mL in chicken semen and $2.4 \times 10^3$ to $1.24 \times 10^6$ cfu/mL in turkey semen (data not shown). Following inoculation with *Campylobacter* and immediately after aeration (0 h), there were no differences in the *Campylobacter* concentrations in semen due to aeration with air or oxygen compared with nonaerated controls (Tables 1 and 2). *Campylobacter* concentrations in chicken semen ranged from $3.8 \times 10^6$ cfu/mL (air) to $6.4 \times 10^6$ cfu/mL (oxygen) compared with $9.3 \times 10^6$ cfu/mL (control) at 0 h (Table 1). Similarly, *Campylobacter* concentrations in turkey semen ranged from $2.5 \times 10^7$ cfu/mL (air) to $3.1 \times 10^7$ cfu/mL (oxygen) compared with $2.6 \times 10^7$ cfu/mL (control) at 0 h (Table 2). Aeration in combination with storage at 4 or 23°C over a 24 h period did not reduce *Campylobacter* concentrations in chicken or turkey semen (Tables 1 and 2). The only reduction in *Campylobacter* concentrations observed in this study was in semen stored at 42°C for 24 h (Tables 1 and 2). However, when stored for 24 h at 42°C, the sperm was no longer viable upon examination.

## DISCUSSION

Introduction of *Campylobacter* into a poultry flock by any source, whether by horizontal or vertical transmission, could lead to rapid dissemination within the flock (Newell and Fearnley, 2003). Any successful strategy to reduce or eliminate *Campylobacter* in poultry production

**TABLE 2. Effects of aeration and storage temperatures on *Campylobacter* concentrations in turkey semen[1]**

| | Control | | | Air | | | Oxygen | | |
|---|---|---|---|---|---|---|---|---|---|
| Time | 4°C | 23°C | 42°C | 4°C | 23°C | 42°C | 4°C | 23°C | 42°C |
| Pretreatment | NA | $3.6 \times 10^{7,a}$ | NA | NA | $3.6 \times 10^{7,a}$ | NA | NA | $3.6 \times 10^{7,a}$ | NA |
| 0 h | NA | $2.6 \times 10^{7,a}$ | NA | NA | $2.5 \times 10^{7,a}$ | NA | NA | $3.1 \times 10^{7,a}$ | NA |
| 2 h | $3.3 \times 10^{7,a}$ | $3.1 \times 10^{7,a}$ | $2.9 \times 10^{7,a}$ | $1.2 \times 10^{7,a}$ | $3.1 \times 10^{7,a}$ | $3.4 \times 10^{7,a}$ | $2.0 \times 10^{7,a}$ | $3.8 \times 10^{7,a}$ | $2.8 \times 10^{7,a}$ |
| 6 h | $4.1 \times 10^{7,a}$ | $3.1 \times 10^{7,a}$ | $1.8 \times 10^{6,a}$ | $4.8 \times 10^{7,a}$ | $3.1 \times 10^{7,a}$ | $1.8 \times 10^{6,a}$ | $4.0 \times 10^{7,a}$ | $3.4 \times 10^{7,a}$ | $2.1 \times 10^{7,a}$ |
| 24 h | $1.6 \times 10^{7,a}$ | $1.2 \times 10^{7,a}$ | $3.3 \times 10^{2,b}$ | $2.2 \times 10^{7,a}$ | $2.0 \times 10^{7,a}$ | $8.0 \times 10^{3,b}$ | $1.5 \times 10^{7,a}$ | $1.6 \times 10^{7,a}$ | $3.3 \times 10^{2,b}$ |

[a,b]Means with no common superscript differ significantly ($P < 0.05$). All data were $\log_{10}$ transformed for statistical analysis. For clarity of presentation, arithmetic means are presented.

[1]Means of 4 separate trials. In each trial, pooled turkey semen was diluted with Field Ready Green Extender (without antibiotics) and inoculated with 1.0 mL of *Campylobacter* enrichment broth containing an average of 8.3 x $10^7$ cells/mL of a wild-type *C. coli* semen isolate. Following inoculation, pooled semen was gently bubbled with atmospheric air using an aquarium pump or with compressed oxygen for 20 min, or not aerated (control). Immediately following the 20 min aeration/control period (0 h), semen samples were allocated to the 4, 23, or 42°C treatment groups. NA = not applicable.

systems will require a multifaceted approach. In an effort to prevent one potential source of *Campylobacter* contamination, the ability of aeration and reduced temperature to eliminate *Campylobacter* in semen was evaluated in 4 trials. Treatments were chosen for their potential to reduce *Campylobacter* concentrations without adversely affecting sperm viability. Unfortunately, in the present study, aerobic treatment of chicken and turkey semen with air or oxygen did not reduce *Campylobacter* concentrations (Tables 1 and 2). Although *Campylobacter* has been reported to be sensitive to aerobic environments (Bolton and Coates, 1983; Koidis and Doyle, 1983), its lack of efficacy in this study may be due to the limited time (20 min) of aeration. Although an extended aeration period may reduce *Campylobacter* concentrations in poultry semen, the impact on sperm viability and farm labor costs would have to be assessed.

The optimum temperature for maintaining the viability of turkey sperm during in vitro liquid storage ranges from 4 to 15°C (Bajpai and Brown, 1964; Sexton, 1982; Blesbois and Mauger, 1989; Christensen, 1995). In contrast, temperatures below 30°C have been shown to be detrimental to the growth and survival of *Campylobacter* (Doyle and Roman, 1981; Hazeleger et al., 1998). When reduced temperatures (4 or 23°C) were evaluated in the present study, these treatments were not effective on *Campylobacter* concentrations in poultry semen (Tables 1 and 2). In addition, the combination of aeration and reduced temperatures, typical procedures used to maintain sperm viability before insemination, did not reduce *Campylobacter* concentrations in vitro. It is unclear why these treatments lacked efficacy. It is possible that these treatments stressed *Campylobacter*, but not to the extent of reducing or eliminating the organism in the pooled semen samples.

The reduction of *Campylobacter* in aerated poultry semen stored for 24 h at 42°C was unexpected. *Campylobacter* is a thermophilic organism that grows best at 42°C (Doyle and Roman, 1981; Ketley, 1997) and this treatment temperature was incorporated into the experimental design as a *Campylobacter* growth control. Although the *Campylobacter* concentrations were reduced after 24 h of storage at 42°C, this approach is not a practical treatment because of reduced sperm viability. One possible explanation for this reduction in *Campylobacter* may be that the pH or osmolality of the semen was altered as the spermatozoa expired. Studies have shown that nutrient media with low environmental pH (Murphy et al., 2003) or low osmolality (Reezal et al., 1998) are detrimental to the survival of *Campylobacter*. Another possible explanation is that as the spermatozoa expired, they released intracellular products that adversely affected the survival of *Campylobacter*. It has been reported that NaCl concentrations as low as 1.0% may inhibit the growth or increase the rate of death of *Campylobacter* (Doyle and Roman, 1982). Another possibility is that in the 6 to 24 h interval, the growth rate of *Campylobacter* was accelerated at 42°C, compared with that at 4 and 23°C, and, in combination with a suboptimal medium (semen extender), the organism consumed the available nutrients and deteriorated to a nonculturable form (Bovill and Mackey, 1997), or the organism expired.

*Campylobacter* is considered to be a fragile organism outside of its living host, requiring specific conditions for growth. In the present study, the aeration and reduced storage temperature of poultry semen were not effective in reducing or eliminating *Campylobacter* contamination. In another study evaluating semen extenders containing various antibiotics, (Donoghue et al., 2004), *Campylobacter* concentrations were reduced, but not eliminated from contaminated turkey semen. It appears that alternative methods will be needed to eliminate *Campylobacter* from poultry semen.

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## REFERENCES

Bajpai, P. K., and K. I. Brown. 1964. The effect of different temperatures on the metabolic activity, morphology and fertilizing capacity of turkey semen. Poult. Sci. 43:1501–1508.

Baker, R. C., J. P. Golf, and J. F. Timoney. 1980. Prevalence of Salmonellae on eggs from poultry farms in New York State. Poult. Sci. 59:289–292.

Blesbois, E., and I. Mauger. 1989. Zinc content of fowl seminal plasma and its effects on spermatozoa after storage at 4 degrees C. Br. Poult. Sci. 30:677–685.

Bolton, F. J., and D. Coates. 1983. A study of the oxygen and carbon dioxide requirements of thermophilic *Campylobacter*. J. Clin. Pathol. 36:829–834.

Bovill, R. A., and B. M. Mackey. 1997. Resuscitation of 'non-culturable' cells from aged cultures of *Campylobacter jejuni*. Microbiology 143:1575–1581.

Buhr, R. J., N. A. Cox, N. J. Stern, M. T. Musgrove, J. L. Wilson, and K. L. Hiett. 2002. Recovery of *Campylobacter* from segments of the reproductive tract of broiler breeder hens. Avian Dis. 46:919–924.

Burrows, W. H., and J. P. Quinn. 1937. The collection of spermatozoa from the domestic fowl and turkey. Poult. Sci. 26:19–24.

Byrd, J. A., R. C. Anderson, T. R. Callaway, R. W. Moore, K. D. Knape, L. F. Kubena, R. L. Ziprin, and D. J. Nisbet. 2003. Effect of experimental chlorate product administration in the drinking water on *Salmonella typhimurium* contamination of broilers. Poult. Sci. 82:1403–1406

Centers for Disease Control and Prevention (CDC). 2002. Preliminary FoodNet data on the incidence of foodborne illnesses — selected sites, United States, 2002. MMWR 52:340–343.

Christensen, V. L. 1995. Diluents, dilution, and storage of poultry semen for six hours. Pages 90–106 in Proc. 1st International Symposium on the Artificial Insemination of Poultry. M. R. Bakst and G. J. Wishart, ed. The Poultry Science Association, Inc., Savoy, IL.

Corry, J. E., and I. Attabay. 2001. Poultry as a source of *Campylobacter* and related organisms. J. Appl. Microbiol. 90:96S–114S.

Cox, N. A., N. J. Stern, K. L. Hiett, and M. E. Berrang. 2002a. Identification of a new source of *Campylobacter* contamination in poultry: Transmission from breeder hens to broiler chickens. Avian Dis. 46:535–541.

Cox, N. A., N. J. Stern, J. L. Wilson, M. T. Musgrove, R. J. Buhr, and K. L. Hiett. 2002b. Isolation of *Campylobacter* spp. from semen samples of commercial roosters. Avian Dis. 46:717–720.

Donoghue, A. M., P. J. Blore, K. Cole, N. M. Loskutoff, and D. J. Donoghue. 2004. Detection of *Campylobacter* or *Salmonella* in turkey semen and the ability of poultry semen extenders to reduce their concentrations. Poult. Sci. 83:1728–1733.

Donoghue, A. M., and G. J. Wishart. 2000. Storage of poultry semen. Anim. Reprod. Sci. 62:213–232.

Doyle, M. P., and D. J. Roman. 1981. Growth and survival of *Campylobacter fetus* subsp. *jejuni* as a function of temperature and pH. J. Food Prot. 44:596–601.

Doyle, M. P., and D. J. Roman. 1982. Response of *Campylobacter jejuni* to sodium chloride. Appl. Environ. Microbiol. 43:561–565.

Friedman, C. R., J. Neimann, H. C. Wegener, and R. V. Tauxe. 2000. Epidemiology of *C. jejuni* infections in the United States and other industrialized nations. Pages 121–138 in *Campylobacter*. I. Nachamkin and M. J. Blaser, ed. ASM Press, Washington, DC.

Gale, C., and K. I. Brown. 1961. The identification of bacteria contaminating collected semen and the use of antibiotics in their control. Poult. Sci. 40:50–55.

Genigeorgis, C., M. Hassuney, and P. Collins. 1986. *Campylobacter jejuni* infection on poultry farms and its effect on poultry meat contamination during slaughter. J. Food Prot. 49:895–903.

Hazeleger, W. C., J. A. Wouters, F. M. Rombouts, and T. Abee. 1998. Physiological activity of *Campylobacter jejuni* far below the minimal growth temperature. Appl. Environ. Microbiol. 64:3917–3922.

Hiett, K. L., N. A. Cox, R. J. Buhr, and N. J. Stern. 2002. Genotype analyses of *Campylobacter* isolated from distinct segments of the reproductive tracts of broiler breeder hens. Curr. Microbiol. 45:400–404.

Jacobs-Reitsma, W. 1997. Aspects of epidemiology of *Campylobacter* in poultry. Vet. Q. 19:113–117.

Jacobs-Reitsma, W. 2000. *Campylobacter* in the food supply. Pages 467–481 in *Campylobacter*. I. Nachamkin and M. J. Blaser, ed. ASM Press, Washington, DC.

Kazwala, R. R., J. D. Collins, J. Hannan, R. A. Crinion, and H. O'Mahony. 1990. Factors responsible for the introduction and spread of *Campylobacter jejuni* infection in commercial poultry production. Vet. Rec. 126:305–306.

Kelana, L. C., and M. W. Griffiths. 2003. Growth of autobioluminescent *Campylobacter jejuni* in response to various environmental conditions. J. Food Prot. 66:1190–1197.

Ketley, J. M. 1997. Pathogenesis of enteric infection by *Campylobacter*. Microbiology 143:5–21.

Koidis, P., and M. P. Doyle. 1983. Survival of *Campylobacter jejuni* in the presence of bisulfite and different atmospheres. Eur. J. Clin. Microbiol. 2:384–388.

Line, J. E. 2001. Development of a selective differential agar for isolation and enumeration of *Campylobacter* spp. J. Food Prot. 64:1711–1715.

McGarr, C., W. R. Mitchell, H. C. Carson, and N. A. Fish. 1980. An epidemiological study of Salmonellae in broiler chicken production. Can. J. Pub. Health 71:47–57.

Murphy, C., C. Carroll, and K. N. Jordan. 2003. Identification of a novel stress resistance mechanism in *Campylobacter jejuni*. J. Appl. Microbiol. 95:704–708.

Newell, D. G., and C. Fearnley. 2003. Sources of *Campylobacter* colonization in broiler chickens. Appl. Environ. Microbiol. 69:4343–4351.

Nielsen, E. M., and N. L. Nielsen. 1999. Serotypes and typability of *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry products. Int. J. Food Microbiol. 46:199–205.

Norkrans, G., and A. Svedhem. 1982. Epidemiological aspects of *Campylobacter jejuni* enteritis. J. Hyg. (Lond.) 89:163–170.

Reiber, M. A., D. E. Conner, and S. F. Bilgili. 1995. *Salmonella* colonization and shedding patterns of hen inoculated via semen. Avian Dis. 39:317–322.

Reiber, M. A., D. E. Conner, S. F. Bilgili, and J. S. Kotrola. 1991. Persistence of *Salmonella typhimurium* and *S. enteritidis* in the oviducts of hens inseminated with contaminated semen. Poult. Sci. 70(Suppl. 1):98. (Abstr.)

Reezal, A., B. McNeil, and J. G. Anderson. 1998. Effect of low osmolality on growth and culturability of *Campylobacter* species. Appl. Environ. Microbiol. 64:4643–4649.

Sahin, O., T. Y. Morishita, and Q. Zhang. 2002. *Campylobacter* colonization in poultry: Sources of infection and modes of transmission. Anim. Health Res. Rev. 3:95–105.

SAS Institute. 1994. SAS/STAT Users Guide. SAS Institute, Inc., Cary, NC.

Sexton, T. J. 1974. Oxidative and glycolytic activity of chicken and turkey spermatozoa. Comp. Biochem. Physiol. B. 48:59–65.

Sexton, T. J. 1982. Short-term holding of turkey semen. Pages 110–113 in Proceedings of the Symposium on Turkey Reproduction, Raleigh, NC.

Sexton, T. J. 1988. Comparison of commercial diluents for holding turkey semen 24 hours at 5°C. Poult. Sci. 67:131–134.

Shane, S. M. 1992. The significance of *Campylobacter jejuni* infection in poultry: A review. Avian Pathol. 21:189–213.

Shivaprasad, H. L., J. F. Timoney, S. Morales, B. Lucio, and R. C. Baker. 1990. Pathogenesis of *Salmonella enteritidis* in laying chickens. I. Studies on egg transmission, clinical signs, fecal shedding and serologic responses. Avian Dis. 34:548–557.

Thurston, R. J., M. S. Rogoff, T. R. Scott, and N. Korn. 1994. Perfluorochemical emulsions as turkey semen diluents: Effects of varying aeration treatments and aqueous phase on fertilizing capacity of semen stored for twenty-four hours. Poult. Sci. 73:724–732.

Timoney, J. F., H. L. Shivaprasad, R. C. Baker, and B. Rowe. 1989. Egg transmission after infection of hens with *Salmonella enteritidis* phage type 4. Vet. Rec. 125:600–601.

Wilcox, F. H., and M. S. Shorb. 1958. The effect of antibiotics on bacteria in the semen and on the motility and fertilizing ability of chicken spermatozoa. Am. J. Vet. Res. 19:945–949.

Wishart, G. J. 1981. The effect of continuous aeration on the fertility of fowl and turkey semen stored above 0°C. Br. Poult. Sci. 22:445–450.

Wishart, G. J., and Y. I. Wilson. 1997. 4. Sperm motility and metabolism. I. Visual scoring of motility using the hang drop method. Pages 46–47 in Techniques for Semen Evaluation, Semen Storage, and Fertility Determination. M. R. Bakst and H. C. Cecil, ed. The Poultry Science Association, Inc., Savoy, IL.

Zhao, C., B. Ge, J. DeVillena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington DC area. Appl. Environ. Microbiol. 67:5431–5436.